REVIEW

Endothelial Integrity, Soluble Adhesion Molecules and Platelet Markers in Type 1 Diabetes Mellitus

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Recent developments in cell biology have identified new areas of direct relevance to the pathogenesis of Type 1 (insulin-dependent) diabetes mellitus and its complications. Endothelial damage is well recognized in diabetes—endothelial cell markers von Willebrand factor, soluble E-selectin, and soluble thrombomodulin are providing further evidence of the relationship between activation and damage to the vasculature and clinical disease in this condition. Cell surface bound adhesion molecules may also have a role in the development of atherosclerosis in patients with diabetes but the importance of the soluble forms of these molecules, such as intercellular adhesion molecule-1, is unclear. Evidence of platelet dysfunction has long been acknowledged in diabetes and new data are discussed. It is likely that a greater appreciation of the intimate interactions between endothelial integrity, adhesion molecules and platelets in Type 1 diabetes mellitus will provide a greater understanding of the risk of cardiovascular disease and stroke in patients with this disorder. © 1998 John Wiley & Sons, Ltd.

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Introduction

Subjects with Type 1 (insulin-dependent) diabetes mellitus (DM) are at increased risk of premature mortality and morbidity, mostly through the development of atherothrombotic vascular diseases such as ischaemic heart disease, peripheral arterial occlusive disease and stroke, and other related clinical complications such as retinopathy, neuropathy and nephropathy. The precise pathogenic mechanisms for these changes are unclear but may involve alterations in several physiological systems, including those of haemostasis, thrombosis, and vascular biology and especially in the correct functioning of the endothelial cell and the platelet.[1–5] Many of these changes are also important in, and relevant to, the pathogenesis of Type 2 (non-insulin-dependent) diabetes mellitus and its complications, but as this has been reviewed in a recent issue of *Diabetic Medicine*,[6] and elsewhere (e.g. Schneider and Sobel[7]), this review concentrates upon Type 1 DM. Although information derived from animal work has also provided useful insights, we primarily consider data collected in man.

Methodology

The ovid medicine database (1990–1997) was searched, using the key words: insulin-dependent diabetes mellitus, endothelial cells, platelets, soluble adhesion molecules. A personal assessment was made of the relevance of each study found to the topic of this review.

The Endothelium

The importance of the endothelium in cardiovascular pathophysiology is well established, and is discussed in several excellent reviews.[6,8–10] The functioning of this organ may be assessed invasively (e.g. by the perfusion of organs with pharmacologically active agents such as acetylcholine), non-invasively (e.g. by using the hyperaemic (flow mediated dilatation) response) and with the use of plasma markers. Vascular biologists have at their disposal a limited number of these specific plasma markers which reflect endothelial cell physiology and which therefore may be used as tools to dissect the disease process.[9,11]

von Willebrand factor

More is known of the physiological (e.g. in exercise) and pathological (e.g. in hypertension) relationships of this molecule than any other marker of endothelial cell damage or dysfunction. Boneu and colleagues were

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among the first to note raised levels in diabetes mellitus although it is unclear whether or not their subjects had Type 1 or Type 2 disease.[12] With even higher levels among patients whose disease was complicated by atherosclerosis, they predicted that raised von Willebrand factor was the result of endothelial damage, a notion now widely accepted.

Tissue culture studies indicate that high glucose levels can cause release of von Willebrand factor from its intracellular storage granule, the Weibel-Palade body, within endothelial cells.[13] Greaves and colleagues showed that resolution of diabetic ketoacidosis may be accompanied by a significant reduction in raised levels of von Willebrand factor and suggested that this may be a mechanism for endothelial damage (Figure 1).[14] Leurs *et al.*[15] found raised von Willebrand factor in Type 1 DM patients, which failed to show a clear relationship with HbA_{1c}. Many groups have shown that increased levels of this marker have a positive relationship with both retinopathy and nephropathy in Type 1 DM, and correlations with urinary albumin excretion are commonly described.[16–21] However, Myrup *et al.* were unable to confirm this relationship, instead finding low HDL-cholesterol[22] and raised sialic acid[23] to have stronger associations with microalbuminuria. Knobl *et al.*[24] found a correlation between von Willebrand factor and albuminuria in Type 1, but not Type 2, DM patients. However, raised von Willebrand factor is not restricted to patients with retinopathy or nephropathy: Plater *et al.*[25] showed that raised levels are associated with deteriorating nerve function, independent of glycaemic control.

The association between von Willebrand factor, endothelial cell injury and Type 1 DM may be extended to include a (possibly causative) relationship with increased systolic blood pressure and oxidant injury, but a relationship with insulin levels is unproven.[26–28] Notably, Stehouwer *et al.*[29] reported that an increase in von Willebrand factor preceded the occurrence of microalbuminuria (but not retinopathy) by approximately 3 years, providing evidence of its value as a potentially useful predictive clinical tool, a finding later confirmed elsewhere.[23] However, Deckert and colleagues showed increased urinary albumin excretion, itself a marker of silent myocardial ischaemia in Type 1 DM,[30,31] to be a better predictor of disease progression than increased levels of von Willebrand factor.[32] However, in this case it cannot be said that the raised von Willebrand factor reflects damage only to the endothelium of the kidney: it is likely to represent a global score of vascular function and is generally unlikely to be a marker of damage to any one specific vascular bed. Hence both raised von Willebrand factor and microalbuminuria would appear to be the result of the same (possibly diabetic) disease process. A similar argument can be applied to the relationship between retinopathy and increased von Willebrand factor: one presumes that the latter is marking endothelial dysfunction, and one aspect of this is damage to the retinal vessels. Increased von Willebrand factor may therefore be the common laboratory measurement of the various mechanisms (e.g. cigarette smoking, free radicals, oxidised lipids) which may bring about endothelial cell injury in a variety of diseases. One consequence of any of these mechanisms may be microalbuminuria, another may be hypertension. It is also possible that high levels of von Willebrand factor take an active part in the disease process by promoting thrombus formation, and it is notable that high levels of this factor predict a poor outcome (e.g. myocardial

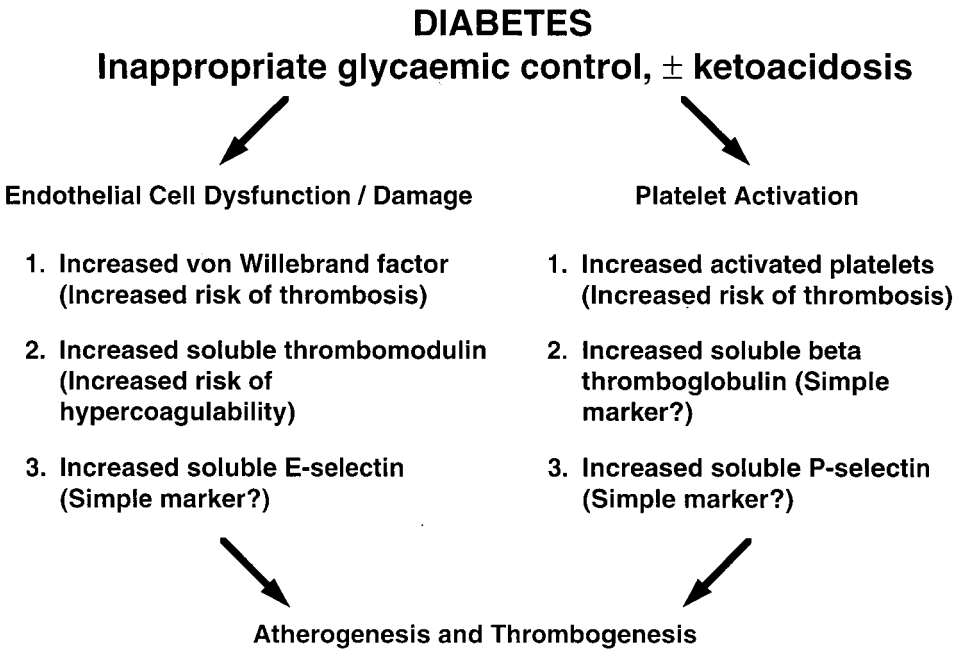

Figure 1. Possible mechanisms to explain the relationship between diabetes and atherosclerosis. Text in parentheses refers to potential participation (or not) in the progression of disease

infarction, stroke, death) in cardiovascular disease.[33,34] We would presume, therefore that the predictive power of this molecule for cardiovascular disease progression is equal in both Type 1 and Type 2 DM, but this needs confirmation in long-term outcome studies.

Patients with microalbuminuria often also have significant elevations of plasma fibrinogen, triglycerides, and LDL-cholesterol, and lower levels of HDL-cholesterol, when compared to controls.[35] Therefore both endothelial and lipid abnormalities add significantly to the complex risk factor profile for cardiovascular disease in Type 1 DM patients.

Soluble Thrombomodulin

Thrombomodulin (CD141) is a proteoglycan component of the endothelial cell membrane with anticoagulant properties. It binds thrombin and converts it into an enzyme that activates the protein C pathway. Consequently, the appearance of a fractionated, soluble form in the plasma implies (by its presumed loss from the endothelial cell surface) not only vascular injury but also a change in haemostasis towards coagulopathy.[36]

Two groups have been unable to find raised levels of this molecule in diabetic subjects with normal albumin excretion rates, but high levels have been found in patients with microalbuminuria and in the presence of clinical nephropathy.[37,38] Gruden *et al*.[39] have also reported higher soluble thrombomodulin in 12 Type 1 DM patients with microalbuminuria when compared to 12 patients with normoalbuminuria. They also found a correlation between soluble thrombomodulin and diastolic blood pressure, a finding similar to that of Gabat *et al*., who also found that soluble thrombomodulin was more prominent in a mixed group (i.e. Types 1 and 2 DM) of hypertensive patients compared to normotensive patients.[40] Leurs *et al*. were initially unable to find raised soluble thrombomodulin, or a clear relationship with HbA_{1c} in 25 Type 1 DM patients free of microvascular complications such as retinopathy compared to 21 healthy controls,[15] but later[41] correlated soluble thrombomodulin with microalbuminuria in a study of 46 patients and 18 healthy controls. Seigneur *et al*.[42] reported the highest levels in diabetic patients with proliferative retinopathy, although it is unclear whether or not their patients had Type 1 or Type 2 DM.

Consequently, there is still considerable scope for research into soluble thrombomodulin in diabetes, especially in outcome and intervention studies. It will be particularly interesting to discover if soluble thrombomodulin levels differ significantly between Types 1 and 2 disease, and if it has more to offer than von Willebrand factor in predicting those patients at increased risk of additional disease. In this respect, for example, McLaren *et al*. reported a preliminary study of raised soluble thrombomodulin, but normal von Willebrand factor, in young (median age 15 years) Type 1 diabetic subjects, suggesting the former is a more sensitive marker of endothelial dysfunction.[43] Hence soluble thrombomodulin represents another opportunity to study vascular function, but more extensive clinical data are required, especially from large studies. There are currently very few follow-up data for soluble thrombomodulin in diabetes, which is eagerly sought as high levels also imply endothelial damage and a procoagulant profile.

Soluble E-selectin

Increased levels of E-selectin (CD62E) appear at the surface of the immunologically activated endothelium, and increased soluble levels also appear in tissue culture supernatants when endothelial cells are co-cultured with cytokines and in the plasma of subjects with certain diseases such as septic shock.[44,45] However, as for soluble thrombomodulin, the definitive clinical experiments that clearly show, in large numbers, a relationship between soluble E-selectin and either Type 1 or Type 2 DM are still awaited. Gearing and colleagues were among the first to find increased levels of soluble E-selectin in (unspecified) diabetes,[45] and several groups have extended this work by showing raised levels in both Types 1 and 2 DM[46,47] although not all workers have agreed with this observation.[48] The latter group was also unable to find increased levels among patients with microangiopathy.

We have been unable to find increased soluble E-selectin among patients with non-diabetic atherosclerosis.[49] It is therefore notable that Kirk and colleagues have reported raised levels in diabetic relative to non-diabetic atherosclerosis and to healthy controls.[50] This suggests that raised soluble E-selectin may have a specific relationship to diabetic vascular biology and not necessarily to atherosclerosis *per se*. Preliminary data from the same group indicates that dietary supplementation with essential fatty acids is effective in reducing increased levels of soluble E-selectin in young people with Type 1 DM.[51] This suggests that it may be possible to restore vascular function with diet manipulation, and so represents a new initiative.

Endothelin

Endothelin is a potent vasoconstrictor derived from the endothelium that acts directly on smooth muscle cells. Its presence in the plasma is presumed to be a spillover from this activity at the interface between the two cell types. Lehman *et al*.[52] hypothesized that plasma endothelin may be an additional marker for arterial vascular disease and speculated that it might be a contributor to atherogenesis or may merely be released from damaged endothelial cells. Takahashi and colleagues were among the first to describe increased levels of plasma endothelin-1 among patients with both Type 1 DM and Type 2 DM.[53] Collier *et al*. have subsequently shown these increased plasma levels are related directly to albuminuria in Type 1 DM[54] while Kawamura *et al*.

have described raised plasma levels in Type 2 DM patients with retinopathy.[55] Curiously, however, levels seem to be *lower* in children and adolescents with Type 1 DM than in their healthy siblings.[56] Smulders *et al.* also found lower plasma levels of endothelin in 10 Type 1 DM adults compared to 10 healthy controls.[57]

Properzi *et al.*[58] examined endothelin in the endothelium of skin biopsies, using immunohistology and electronmicroscopy. They found levels of tissue endothelin-1 to be higher (present in 82 % of cells in the biopsies) in patients with Type 1 DM of less than 10 years duration, than in healthy controls, where only 50 % of cells were stained. However, only 17 % of cells from patients with long standing diabetes were reactive, and the percentage of biopsies showing positive endothelin-1 staining was lower in patients with retinopathy than in patients without retinopathy. No differences in staining for von Willebrand factor were observed. It has been suggested that measurement of plasma endothelin may provide some useful information, especially as a vascular marker,[59,60] and a thorough comparison with other endothelial markers such as von Willebrand factor would be valuable. However, Gruden *et al.*[61] have been unable to find raised plasma endothelin in 13 microalbuminuria patients compared to 13 normoalbuminuric patients.

Therefore at present there seems to be little consensus regarding a role for plasma endothelin in Type 1 DM. The likelihood of false positives and false negatives in the small studies described can only be addressed by studies in large (>100) numbers of patients with age- and sex-matched controls. The relationship between plasma endothelin and insulin also needs to be fully explored.

Other Endothelial Products

Vascular endothelial cells synthesize an inhibitor of tissue factor (a co-factor for coagulation factor VIIa) called tissue factor pathway inhibitor, or TFPI, and may be its principal source.[62–64] It is unclear whether or not raised levels of this anticoagulant reflect endothelial cell damage, but increased TFPI (defined by a chromogenic, not a clotting, assay) is found in Type 1 DM, correlating with glycated haemoglobin but not with increased von Willebrand factor.[15] A subsequent report from the same group[41] showed higher levels in patients with microalbuminuria compared to patients with retinopathy or patients without microangiopathic complications. Yokayama *et al.*[65] found that plasma TFPI correlated with urinary albumin excretion (which may be considered a surrogate marker of vascular damage) in 59 patients with Type 1 DM.

Changes in levels of other molecules likely to be important in thrombosis and haemostasis have also been reported. Leurs *et al.*[15] found low levels of tissue plasminogen activator (tPA) in diabetes, but no change in levels of its inhibitor (i.e. plasminogen activator inhibitor (PAI)). Skrha *et al.*[66] found raised tPA regardless of degree of retinopathy, and Gruden *et al.*[61] found tPA to be uninfluenced by albumin excretion rate, although this group found PAI to be higher in subjects with microalbuminuria. However, the precise value and status of tPA and PAI as specific endothelial cell markers are unclear. For example, they can be measured by immunological or functional methods, and may form a (possibly inactive) complex *in vivo* and/or *in vitro*. Furthermore, some may derive from non-endothelial sources such as platelets.[67–70] An example of the difficulty in interpretation of levels of tPA is the paradox that smoking, widely believed to be pathogenic towards the endothelium, the platelet, and to increase soluble coagulation factors, increases the activity of plasma tPA,[71] which should promote clot dissolution, and so reduce the risk of thrombosis.

Soluble Adhesion Molecules

In addition to endothelial cell soluble E-selectin already described, several other soluble adhesion molecules have been studied in Type 1 DM: these are intercellular adhesion molecule-1 (ICAM-1, CD106), vascular cell adhesion molecule-1 (VCAM-1, CD54), soluble L-selectin (CD62L), and soluble P-selectin (CD62P). The first two are both members of the immunoglobulin gene superfamily, the last two will be discussed in the section on platelets which follows. Raised soluble L-selectin has been described in Type 1 DM,[72] but there is little other information about this molecule in diabetes which can, in other circumstances, reach levels high enough to inhibit cell to cell adhesion.[75] Least is known about the most recently characterized, platelet–endothelial cell adhesion molecule-1 (PECAM-1, CD31).[73] Levels do not seem to be altered in atherosclerosis[74] and a preliminary report found levels in diabetes to be no different from those in healthy controls.[76] For more comprehensive recent reviews on the cell biology of adhesion molecules (such as their involvement in cell rolling and adhesion) see Carter and Grant,[6] Jang *et al.*,[77] Kansas,[78] and Frenette and Wagner.[79]

Soluble ICAM-1

This adhesion molecule is a transmembrane glycoprotein that binds to members of the beta 2 integrin family; leucocyte function associated antigen (CD11a/CD18) and Mac-1 (CD11b/CD18), and to the sialophorin CD43.[80] Gearing *et al.* were among the first to describe raised plasma levels in diabetes,[45] and Lampeter *et al.* reported increased levels in 4 out of 14 patients with recent onset Type 1 DM and in first degree relatives with a genetic predisposition to the disease.[72] They postulated from their subsequent *in vitro* data that soluble ICAM-1 may downregulate inflammatory responses, providing a novel opportunity to intervene in the disease process.[81] Interestingly, therapy with monoclonal anti-

bodies to ICAM-1 prevents graft rejection and Type 1 DM recurrence in a rat model.[82] Other animal work has led Yagi *et al*. to suggest that ICAM-1 plays an important role in the pathogenesis of Type 1 DM via involvement in the extravasation of lymphocytes from the plasma into the inflamed pancreas.[83] Whether or not soluble ICAM-1 modulates this process in humans remains to be proven.

However, an alternative view is suggested by the data of Accardo-Palumbo *et al*.,[84] who found higher soluble ICAM-1 in patients who were positive for antibodies to myeloperoxidase. They postulated that damage to the endothelium (as suggested by raised soluble ICAM-1) is due to chronic neutrophil activation by antibodies to myeloperoxidase. Both these avenues are worthy of additional study, especially as Fasching *et al*.[48] have correlated raised levels of soluble ICAM-1 with the presence of microangiopathy, although Cominacini *et al*. have not been able to find raised levels of ICAM-1 in Type 1 DM.[47]

Esser and colleagues have taken a novel approach. They reported ICAM-1 to be raised by 13 % in the plasma of Type 1 DM patients with proliferative diabetic retinopathy (PDR), but by only 2 % in the plasma of Type 2 DM patients with PDR.[85] However, they also measured levels in vitreous humour from the patients, finding levels to be about 1 % of those in healthy control plasma. In the Type 1 DM patients with PRD, levels were 5.8 % of those in the plasma, while in Type 2 disease, levels were 7.6 % of paired plasma levels. The highest levels relative to plasma were in idiopathic (8.9 %) and in traumatic PDR (10.4 %). They conclude that local ICAM-1 production, possibly from macrophages, may be of importance in the early phase of retinal disease by enhancing immune activation and inflammation.

Soluble VCAM-1

This molecule, also a member of the immunoglobulin gene superfamily, is found on endothelial cells and dendritic cells, and weakly raised levels in diabetes have been reported.[45] Fasching *et al*. also described higher levels in 37 patients with Type 1 DM but no microangiopathy than in 70 healthy controls. However, levels were even higher among 33 patients who had microangiopathy compared to the Type 1 patients who were free of microangiopathy.[48] However, as for soluble ICAM-1, Cominiacini *et al*.[47] did not find levels of soluble VCAM-1 to be higher in 18 patients with Type 1 DM compared to 20 healthy controls. These discrepancies clearly need to be resolved in larger studies, as they may have implications for vascular biology and the possible use of antibody therapy directed towards the cell surface from of the adhesion molecule. For example, administration of monoclonal antibodies to VCAM-1 inhibited the development of insulitis and diabetes in a mouse model of Type 1 DM.[86]

A recent exciting report has been the preliminary data from Abeygunaratne *et al*.[87] They showed that increased soluble ICAM-1, but not soluble VCAM-1 or soluble E-selectin, predicted the development of vascular complications in 11 of 36 people (17 with Type 1 DM, 19 with Type 2 DM) followed up for 5 years. The full report of this study will provide evidence of an additional tool to discover those subjects at risk of additional disease.

Platelet Markers

Platelets have long been implicated in the pathogenesis of the vascular disease that accompanies Type 1 DM,[88] reviewed in Colwell *et al*.[4,89] The alpha granule component, beta thromboglobulin (Figure 1), has been widely used to look for inappropriate platelet activity in Type 1 DM and its complications.[90–95] Patrick *et al*.[90] concluded that urinary beta thromboglobulin is not sensitive enough to be useful in the detection of early renal disease, despite raised levels being found in diabetes. Rak *et al*.[93] found that plasma levels closely correlated with the progression to angiopathic disease, although most of their cohort had Type 2 DM and Bayraktar *et al*.[94] were unable to find raised levels of beta thromboglobulin in 30 patients with Type 1 DM compared to 15 controls. More recently Huszka and colleagues[95] reported levels of 169.1 ± 44.6 ng ml^{-1} (mean ± SD) in 15 patients with Type 1 DM, 65.2 ± 18.4 ng ml^{-1} in 35 patients with Type 2 DM, compared to their non-diabetic reference range of 15–35 ng ml^{-1}. The differences in these values would appear to be significant, but no statistical analyses were presented. There is a further report that subjects with Type 1 DM have raised beta thromboglobulin[6] and the longitudinal study has shown the marker to be a significant predictor of disease progression.[96] Despite this interest, it is by no means clear that uncomplicated Type 1 DM is associated with platelet activation.

Soluble P-selectin (CD62P) has recently been proposed as a new marker of platelet activation.[97,98] Being shed from the platelet membrane, this adhesion molecule provides an additional perspective to the matrix alpha granule component beta thromboglobulin. Raised levels of both markers are found in non-diabetic atherosclerosis[99] and increased soluble P-selectin has been reported in Type 1 DM.[100] However, it is not yet known whether there are differences according to retinopathy or nephropathy and a good comparison with beta thromboglobulin would provide much needed data. More recently, Rauch *et al*. have used membrane bound P-selectin to provide preliminary data indicating increased numbers of circulating activated platelet in Type 1 DM patients relative to healthy controls.[101] They interpreted lower levels of such activated cells in patients with microangiopathy as evidence of increased consumption. Conversely, Tschoepe *et al*. found higher numbers of platelets bearing P-selectin in prediabetic subjects, concluding that these subjects have platelet activation which may represent an antecedent, potentially pathogenic feature of Type 1 DM.[102]

Conclusions and Speculations

Physiological techniques for assessing endothelial function in diabetes (such as invasive, pharmacological methods, and the non-invasive flow mediated dilatation studies,[103] are impractical for large epidemiological studies of the aetiopathogenesis of the complications of Type 1 DM. Several plasma molecules, markers of endothelial (dys)function, are available which may be useful in extending our knowledge of the pathophysiology of diabetes. For some, interesting preliminary data have been published but they are often derived from small numbers of subjects and/or from studies of cross-sectional design. In others the lack of a definition of the precise type of diabetes of the patients has lead to difficulties in interpretation. A further difficulty with the interpretation of increased levels of many of these markers (such as soluble ICAM-1) is that they may simply relate to the disturbances in autoimmunity and not necessarily to the insulin/glycosylation aspects of the disease.

Adequate endothelial cell functioning is as crucial in Type 1 DM as it is in other conditions.[9,104] In this respect, there is little doubt that von Willebrand factor has most to offer as a marker of this process. As for platelets, although both beta thromboglobulin and soluble P-selectin are increased, so only the former has been described as a significant predictor of disease progression.[96] Although unlikely to be of value as diagnostic aids, students of the endothelium and platelet will be interested to learn if any of the markers behave differently in the two types of diabetes as this may provide clues to the interaction between the disease processes and the biology of the cell concerned.

Whether or not soluble adhesion molecules are simple markers of various physiological and pathological processes remains to be proven. They may perhaps help provide clinical guidance as to those patients at risk of the development of cardiovascular complications, and may prove to have relevance to cell/cell adhesion *in vivo*. It seems likely that a large fraction of the levels of certain plasma markers, such as tPA and soluble ICAM-1, arises from the endothelium. However, this may be of little interest to the clinician unless they can provide useful information about current disease activity and/or the risk of developing complications. It is possible that the increasing interest in the platelet and endothelium as likely participants in pathogenesis[3,4,105–107] may translate into the desire to direct treatment in this direction. Anti-platelet (e.g. aspirin) and anticoagulant (e.g. Warfarin) drugs for the former are established, but for the endothelium, only antihypertensives (such as ACE inhibitors) may directly act on the endothelium, and as they are common in clinical practice they may represent a possible growth area for those interested in retaining and improving vascular integrity.

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